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Research Article

ANALYTICAL STUDY OF *BRAHMI GHRTIA*: A POLYHERBAL AYURVEDIC COMPOUND

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ABSTRACT

Brahmi Ghrita was processed as per the process of *Snehapaka* procedure described in classics. It contained *Brahmi* (*Bacopa monneri*), *Sunthi* (*Zingiber officinale*), *Maricha* (*Piper nigrum*), *Pippali* (*Piper longum*), *Shyama* (*Operculina turpethum*), *Trivrit* (*Operculina turpethum), Danti (Baliospermum montanum), Sankhpuspi (Convolvulus pluricaulis), Nrpadruma (Cassia fistula), Saptala (Euphorbia dracunculoides),* and *Vidanga (Embelia ribes),* were mixed in *Ghrita* and heated for three hours at 110°C every day for three days. On the third day *Ghrita* was filtered to obtain the finished product. In this manner, three samples of *Brahmi Ghrita* were prepared. To understand the changes that occurred during the preparation, *Brahmi Ghrita* were analysed by using modern parameters such as acid value, saponification value, and so on. After the analysis, it was found that the Rancidity were absent in *Brahmi Ghrita*; acid values of *Brahmi Ghrita* were 1.81; the saponification values were 212.30; the iodine values were 39.17; the refractive index was 1.4590; the moisture were 0.12%; and the specific gravity were 0.9441 respectively. The present study revealed that, there was no significant variation in the analytical values among all three samples of *Brahmi Ghrita*. TLC was carried out after organizing appropriate solvent system in which maximum 6 spots were distinguished.

INTRODUCTION

Brahmi Ghrita, a traditional Ayurvedic formulation, has long been revered for its profound therapeutic benefits, particularly in enhancing cognitive function, reducing stress, and promoting overall mental well-being[1]. *Brahmi* being a *Medhya* drug is recommended for a variety of psychosomatic and psychiatric disorders. The majority of formulations that effect on the psyche is ghee based. It is well established that, the drugs to have its action on brain should have the capacity to cross the blood–brain barrier and for that purpose *Ghrita* is most significant drug vehicle. In the present study, the selected *Brahmi Ghrita* contained *Brahmi* (*Bacopa monneri*), *Sunthi* (*Zingiber officinale*), *Maricha* (*Piper nigrum*), *Pippali* (*Piper longum*), *Shyama* (*Operculina turpethum*), *Trivrit* (*Operculina turpethum), Danti (Baliospermum montanum), Sankhpuspi (Convolvulus pluricaulis), Nrpadruma (Cassia fistula), Saptala (Euphorbia dracunculoides), Vidanga (Embelia ribes),* and *Go-Ghrita* mentioned for the treatment of *Unmada, Kushtha*, *Apasmara* (epilepsy), infertility, enhance cognitive function, improve speech, improve memory and concentration[2]. It is an important formulation mentioned in *Ashtang Hridya* as well as in many others classical books of Ayurveda, with different compositions, for the treatment of different disorders.

The standard of quality of any medicine is quite important for the reproducibility of the therapeutic effect. Different types of Ayurvedic medicine criteria are being prepared by certain organizations. The Central Council of Research in Ayurveda and Siddha has published a standard protocol, wherein analytical parameters must be followed for the quality production of Ayurveda medicine.

Brahmi Ghrita is prepared by heating *Brahmi Swarasa* with *Kalka*, made with *Brahmi*, *Sunthi*, *Maricha*, *Pippali*, *Shyama*, Trivrit*, Danti, Sankhpuspi, Nrpadruma, Saptala, Vidanga,* and *Go Ghrita*, in the prescribed quantity. According to *Vaidyak Paribhasha Pradeep*, it is mentioned that when *Swarasa* is used in the *Snehapaka* process, the *Snehapaka* must be completed in three days. the first two days are initially heated for three hours, and the third day is heated till

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the *Sneha Siddhi Lakshana* is accomplished. The chemical changes that occur during the process on account of heating the *Kalka Dravya*, liquid, and *Go Ghrita*, are observed in the present study. This would help us to determine the efficacy of *Brahmi Ghrita* for the above-cited therapeutic purpose.

AIMS AND OBJECTIVES

- Identification and authentication of raw drugs used for *Brahmi Ghrita.*
- Preparation of *Brahmi Ghrita* at GMP-certified pharmacy.
- Organoleptic characters, physicochemical and TLC analysis of *Brahmi Ghrita.*

MATERIAL AND METHODS

All drugs were collected from the local raw drug market of Jodhpur and *Go-Ghrita* was collected from a village near Jodhpur.

Preparation of *Brahmi Ghrita*

In the process of preparing *Brahmi Ghrita*[3], *Brahmi Swarasa* was employed as the *Drava Dravya*, while *Brahmi*, *Sunthi*, *Maricha*, *Pippali*, *Shyama*, *Trivrit, Danti, Sankhpuspi, Nrpadruma, Saptala, Vidanga* was utilized to prepare *Kalka.* As *Brahmi Swarasa* is one of the liquid ingredients mentioned in the reference, as per the general protocol of *Bhaishjya Kalpana, Brahmi* was also included in the *Kalka Dravyas* together with the other ingredients, namely *Sunthi*, *Maricha*, *Pippali*, *Shyama*, *Trivrit, Danti, Sankhpuspi, Nrpadruma, Saptala, Vidanga.* This *Kalka* was then combined with *Brahmi Swarasa* and *Go-Ghrita,* and it was heated to a moderate temperature (about 110°C) till *Sneha Siddhi Lakshana* was noticed, such as *Sabdahinoagni-nikshipto, Vartivat Sneha Kalka*, and so forth [4]. When the *Sneha Siddhi Lakshna* appeared then the *Ghrita* was filtered and *Brahmi Ghrita* was procured.

Analytical study

After preparation of *Brahmi Ghrita,* it was sent to Cultivator Phyto Lab Private Limited, Jodhpur, Rajasthan. The following result was obtained after analysis.

Organoleptic characters

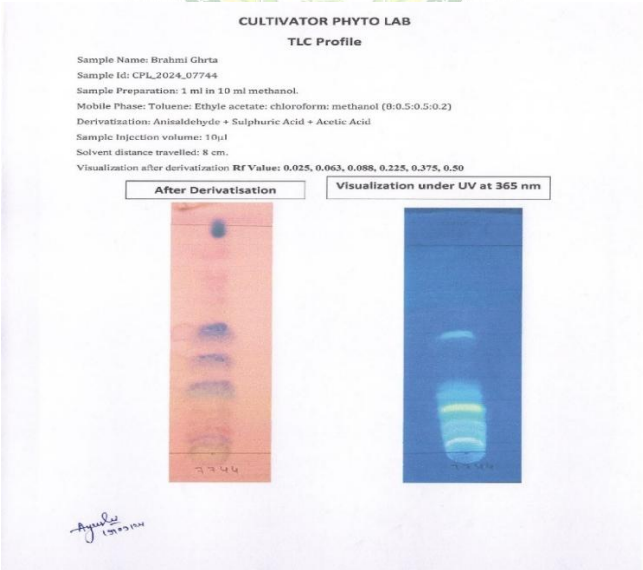
Color: Algae green
Odor: Characteristic (of ghee)
Appearance: Semisolid
Test: Bitter

Phyto-chemical parameters

S.No.	Parameters	Result
1.	Rancidity	Absent
2.	Saponification value	212.30
3.	Iodine value	39.17
4.	Refractive index	1.4590
5.	Acid value	1.81
6.	Moisture	0.12%
7.	Specific gravity	0.9441

Thin layer chromatography

Thin Layer Chromatography (TLC) of *Brahmi Ghrita* was performed using a sample preparation method where 1ml of *Brahmi Ghrita* was dissolved in 10mL of methanol. The mobile phase consisted of a mixture of toluene, ethyl acetate, chloroform, and methanol in a ratio of 8:0.5:0.5:0.2. After preparing the sample and the mobile phase, a 10µl volume of the sample was carefully injected onto the TLC plate. The plate was then developed in the mobile phase, allowing the solvent to travel a distance of 8cm. Following the development, the TLC plate was subjected to derivatization using a mixture of anisaldehyde, sulfuric acid, and acetic acid. Upon heating, the plate was visualized, revealing distinct spots corresponding to various components in the *Brahmi Ghrita* sample. The Rf values for these spots were observed at 0.025, 0.063, 0.088, 0.0225, 0.375, and 0.50 at 365nm, which could be indicative of the presence of different bioactive compounds within the formulation. This TLC profiling provides useful insights into the chemical composition of *Brahmi Ghrita*.


CULTIVATOR PHYTO LAB
TLC Profile
Sample Name: Brahmi Ghrta
Sample Id: CPL_2024_07744
Sample Preparation: 1 ml in 10 ml methanol.
Mobile Phase: Toluene: Ethyle acetate: chloroform: methanol (8:0.5:0.5:0.2)
Derivatization: Anisaldehyde + Sulphuric Acid + Acetic Acid
Sample Injection volume: 10µl
Solvent distance travelled: 8 cm.
Visualization after derivatization Rf Value: 0.025, 0.063, 0.088, 0.225, 0.375, 0.50
After Derivatisation
Visualization under UV at 365 nm

DISCUSSION

Organoleptic analysis

Organoleptic evaluation is a sensory assessment of a substance based on its physical characteristics, including its appearance, texture, colour, odour, and taste. Organoleptic evaluation serves several functions in the preparation of *Brahmi Ghrita*. It ensuring that that the preparation has a consistent appearance, texture, and taste is vital for determining its quality. Variations in these parameters might suggest batch-to-batch inconsistencies or a deviation from standard Ayurvedic practices [5].

Rancidity

Rancidity in *Brahmi Ghrita*, primarily caused by the oxidation and hydrolysis of *Ghrita*, significantly impacts its sensory qualities and therapeutic efficacy. Proper storage, packaging, and quality control

measures are crucial to prevent rancidity and ensure the formulation maintains its potency and effectiveness. Regular organoleptic and chemical testing can help identify early signs of spoilage and ensure that *Brahmi Ghrita* remains safe and beneficial for consumption [6].

Saponification value

The saponification value of long-chain fatty acids, which are found in fat, is low, whereas that of short-chain fatty acids (SCFAs) is high[7]. It has been demonstrated that short-chain fatty acids are a vital source of energy for colonocytes, especially those in the distal colon[8]. The histological, endoscopic, and metabolic similarities between diversion colitis and ulcerative colitis suggest that a nutritional SCFA-deficiency state may play a role in the pathogenesis of these disorders.

Short chain fatty acids are readily absorbed; there may be a protective benefit if SCFA production is increased and SCFAs, particularly butyrate, are delivered to the distal colon more effectively[9]. *Brahmi Ghrita* has a higher saponification value. Fatty acid (short chain) is produced when *Ghritas*, which are esters, hydrolyse in the presence of an alkali (due to the alkaline nature of *Kalka Dravya* or the other *Drava Dravya* used in the *Snehapaka* procedure). This indicates that *Brahmi Ghrita* has more short-chain fatty acids. Therefore, *Brahmi Ghrita* is easily absorbed and digested, has a preventive effect, and enhances intestinal and systemic health.

Iodine value

The amount of unsaturated fatty substance in the *Ghrita* is determined by the iodine levels. The amount of unsaturated bonds in the fat increases with the iodine number. Unsaturated fat supplementation increases overall dietary energy intake to the necessary amounts without negatively affecting blood lipid levels. It also improves nutritional status and lowers systemic inflammation. Polyunsaturated fatty acids, which provide health benefits like controlling blood cholesterol levels, are abundant in lipids with a high iodine value[10]. The increased iodine value of *Brahmi Ghrita* suggests that it contains more unsaturated fatty acids. This analytical value demonstrates that *Brahmi Ghrita* improves nutritional status and lowers systemic inflammation without having a negative effect on blood lipids despite the fatty acid. The increasing unsaturation of the *Ghrita* can be the result of the *Snehapaka* process.

Refractive index

The refractive index is an essential optical property that quantifies how light is distorted as it travels through a material. In the context of *Brahmi Ghrita*, the refractive index can reveal valuable insights into the composition, purity, and quality of the formulation. This characteristic can be particularly helpful in determining the uniformity of ingredients, such as the lipid matrix of *Ghrita* and the active compound in *Brahmi* (bacosides), and comprehending how they interact in the finished product [11].

One key aspect of measuring the refractive index is its role in quality control. Studies has demonstrated that the refractive index of oils and fats, such as ghee, can serve as an indicator of purity and adulteration[12]. Variations in the refractive index of *Brahmi Ghrita* may indicate the presence of impurities like too much water, artificial additives, or inferior raw ingredients. Therefore, maintaining the formulation's intended therapeutic qualities would depend in large part on the refractive index of *Brahmi Ghrita* being consistent throughout batches.

Acid value

The acid value, which is related to the stability of the *Ghrita*, indicates the amount of free fatty acid (FFA) in the *Ghrita*. For the *Ghrita*, the production of free fatty acids may be a crucial indicator of rancidity. Triglycerides hydrolyse to produce FFA, which can be accelerated by the *Ghrita's* contact with moisture [13]. The *Ghrita's* stability, flavour, and shelf life are all impacted by its fatty acid composition. The presence of FFA in the *Ghrita* signifies its purity or individuality [14]. The acid value of *Brahmi Ghrita* is greater. This suggests that *Ghrita* undergoes hydrolysis during the *Snehapaka* process, which could be aided by the reaction of the *Ghrita's* triglycerides with the active components of *Brahmi Ghrita*, which produces glycerol and free fatty acids. Excessive levels of free fatty acid (acid value) encourage a decline in *Ghrita* quality. This demonstrates that *Brahmi Ghrita's* nutritional value, stability, and shelf life is inferior to those of *Go Ghrita*.

Moisture

The moisture content in *Brahmi Ghrita* plays a vital role in determining its shelf life, stability, and therapeutic effectiveness. The base component used to make *Brahmi Ghrita* is usually *Ghrita*, which has a low moisture level (less than 0.2%). The presence of moisture can lead to the growth of microbial organisms, reducing the shelf life of the product. Research on other Ayurvedic formulations, such *Chyawanprash*, has demonstrated a correlation between moisture content and spoiling and microbial development [15]. Excess moisture can alter the balance of ingredients and possibly affect the pharmacological properties of *Brahmi Ghrita*. For instance, *Bacopa monnieri* contains bioactive compounds like bacosides that are susceptible to deterioration under inappropriate storage conditions. Moisture could potentially compromise the bioavailability of these compounds [16].

Specific Gravity

The density of one substance divided by the density of a reference substance, typically water, is known as specific gravity. For liquids and semi-solid preparations such as *Brahmi Ghrita*, the specific gravity provides information about the relative density and purity of the formulation. Since both *Ghrita* and *Brahmi* extracts play a role in the composition of *Brahmi Ghrita*, changes in specific gravity could indicate variations in their concentrations or the presence of contaminants, which can affect the therapeutic efficacy [17].

TLC (Thin layer chromatography)

The key active compounds in *Brahmi Ghrita*, particularly bacosides (Bacopa saponins), are responsible for its therapeutic properties, which include cognitive enhancement and neuroprotection. TLC can be used to separate and identify these compounds based on their Rf (retention factor) values, which are unique for each compound. In a study by Ghosal et al. (2000), TLC was used to isolate bacosides A and B from *Bacopa monnieri* extracts. The Rf values of these compounds were compared with known standards to confirm their presence in the herbal extract[18]. TLC is frequently used in the herbal and pharmaceutical industries for quality control. For *Brahmi Ghrita*, TLC can serve as a tool to monitor batch-to-batch consistency and anticipate the presence of the expected bioactive compounds. According to a study by Kaur et al. (2018) demonstrated that TLC was an effective method for analysing *Chyawanprash* (another Ayurvedic formulation) for its active principles, ensuring its consistency. By applying similar methods to *Brahmi Ghrita*, TLC can help confirm that each batch meets the desired specification for active compounds, ensuring therapeutic efficacy[19]. TLC can be used to detect unwanted substances such as microbial contamination, solvent residues, or adulterants that may affect the quality and safety of *Brahmi Ghrita.* In contrast to the target compounds expected Rf values, impurities may show up on the TLC plate as extra spots. In a study by Gawande et al. (2017), TLC was employed to monitor the purity of Ayurvedic *Ghrita*-based formulations, helping to identify potential contaminants and adulterants [20]. A similar approach can be applied to *Brahmi Ghrita* to ensure that it remains free of foreign substances that could compromise its safety or effectiveness. The "fingerprint" refers to the distinctive pattern of spots produced by the compounds present in the sample when subjected to TLC. This fingerprint can be used to compare samples of *Brahmi Ghrita* from various batches or even from different manufacturers to ensure authenticity and consistency [21].

CONCLUSION

Pharmacognostical study findings confirm the ingredients present in the *Brahmi Ghrita*. Identified phytochemical components like bacoside support the intended action of the formulation. It is inferred that the formulation meets maximum qualitative standards. Under densitometer under 365nm 6 peaks were found. The results of this study may be used as the reference standard in further research undertakings of its kinds.

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